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Uncovering genetic correlations between traits crucial to population dynamics

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Abstract

Per-capita growth rate at low population density and population size at equilibrium are two essential demographic parameters known as intrinsic rate of increase and carrying capacity, respectively. These parameters have been assumed to trade off with each other. Nonetheless, the limited empirical evidence for this trade-off has motivated the formulation of alternative models, stating that *r* and *K* could actually be positively correlated or not correlated at all. These models rest upon the assumption that traits underlying *r* and *K* partially overlap. Given these conflicting theories, we investigate empirically how the intrinsic rate of increase and the carrying capacity are correlated and how they are affected by underlying traits. To this aim, we used 17 isofemale lines of *Tetranychus urticae*, in which we measured: 1) the intrinsic rate of increase (*r*), through the Euler-Lotka equation using age-specific fecundity and survival of the females; 2) the carrying capacity (*K*) as the female density could be sustained by a given amount of resource. Both demographic parameters presented genetic variation among them, however no correlation was found. We also measured the genetic variation in traits that are potentially associated with *r* and *K*: (i) the resource use – measured through the percentage of area of damage done by one individual while feeding; and (ii) the competitive interference – obtained as proportion of how the resource use of a focal individual is affected by the presence of competitors. Both of these traits presented genetic variation and were correlated with each other, however no correlation was found with the demographic parameters. These results might indicate that *Tetranychus urticae* may benefit from not being specialized in any of the demographic parameters, since in a natural environment this species tend to go through a scenario more favorable to an exponential population growth and later a scenario characterized by limited resources.

Keywords: Population dynamics; intrinsic rate of increase; carrying capacity; genetic correlations.

Resumo

A taxa de crescimento intrínseco (*r*) é um parâmetro demográfico usado para descrever a capacidade de uma população crescer em condições ambientais ótimas, sem restrições. Este parâmetro demográfico procura caraterizar o crescimento de cada indivíduo da população, sem ter em consideração os efeitos do aumento da densidade. Desta forma, a taxa de crescimento *per capita* mantêm-se independentemente do tamanho da população, fazendo com que a população cresça mais rápido à medida que aumenta em tamanho. Este tipo de dinâmica é caraterizado por um crescimento exponencial da população. No entanto, os recursos do meio ambiente (por exemplo, a quantidade de alimentos e o espaço disponível) são limitados e vão acabar por afetar de modo negativo a reprodução e a sobrevivência dos indivíduos da população, resultando num crescimento populacional dependente da densidade. De facto, à medida que os recursos se tornam limitantes, uma determinada densidade populacional vai ser atingida, na qual o ambiente não suporta mais indivíduos. Nessa altura, a taxa de mortalidade iguala a taxa de natalidade. Ao número máximo de indivíduos que um ecossistema consegue sustentar é chamado capacidade de suporte (*K*).

Em ambientes naturais, as populações podem apresentar uma fase de crescimento exponencial por alguns períodos, mas geralmente o crescimento irá ser limitado pela disponibilidade de recursos presentes no ambiente. Desta forma, o crescimento *per capita* vai diminuindo à medida que o tamanho da população se aproxima do máximo imposto pelos recursos no ambiente, que é a designação de outro parâmetro demográfico, a capacidade de suporte. De acordo com esta equação, uma população apresenta um crescimento exponencial nas fases iniciais do crescimento, indo subsequentemente reduzindo a taxa de crescimento até atingir um ponto de equilíbrio, em que a população não cresce nem decresce. Visto que ambos *r* e *K* descrevem a dinâmica de uma população, é essencial compreender como é que estes se correlacionam um com o outro. No entanto, esta relação foi raramente testada empiricamente. Além do mais, muitos estudos de ecologia acerca da dinâmica de populações assumem que as populações são constituídas por indivíduos idênticos. No entanto, estudos recentes têm demonstrado que a variação individual pode afetar diversos parâmetros ecológicos. Esta variação pode então influenciar a correlação entre os parâmetros demográficos, mas tal nunca foi testado.

Diferentes teorias tentam explicar de que modo *r* e *K* se relacionam. Uma delas é a teoria *r* e *K*, que pressupõe a existência de dois ambientes contrastantes que moldam as populações de maneira oposta. Assim, algumas populações encontram-se em ambientes-*r*, caraterizados por serem imprevisíveis e com poucos competidores. Desta forma, a capacidade de suporte nunca é atingida. Devido às caraterísticas erráticas do ambiente, prevê-se que características reprodutivas, como o desenvolvimento rápido ou a alta fecundidade (características que levam a um *r* maior) sejam favorecidas, em detrimento de características relacionadas com maior sobrevivência, como o tamanho corporal ou a longevidade. Por outro lado, num ambiente maioritariamente estável, as populações selecionadas para *K* vivem próximas da sua capacidade de suporte e o seu crescimento é altamente dependente da densidade. Nestas condições, é favorecido o investimento em caraterísticas que favoreçam a sobrevivência, como a capacidade competitiva, tamanho e longevidade, em detrimento do investimento em aspetos reprodutores. Desta forma, a teoria *r* e *K* sugere a existência de uma correlação negativa entre os dois parâmetros populacionais. Uma segunda hipótese admite que o *r* e o *K* possam estar correlacionados de uma forma positiva. De facto, através da decomposição da equação logística, podemos perceber que *K* pode estar correlacionado de forma positiva com r ou pode variar independentemente de *r*, exibindo uma ausência de correlação.

Para compreender se existe variabilidade genética para estes dois parâmetros demográficos e para entender a forma como se relacionam foram usadas 17 linhas iso-fêmeas de *Tetranychus urticae.* Esta espécie apresenta ser um modelo ideal para este estudo por diversas razões. Primeiro, porque populações naturais desta espécie são esperadas deparar-se com ambos os ambientes descritos pela teoria *r* e *K*. Inicialmente, estes ácaros-aranha migram para plantas desinfestadas (sem a presença de competidores) onde rapidamente começam uma nova população (ambiente-*r*). No entanto, após algumas gerações, as

populações são constituídas por altas densidades de indivíduos levando a uma saturação do ambiente (ambiente-*K*), o que leva aos indivíduos migraram para uma nova planta onde o processo repete-se. Por fim, esta espécie apresenta ser um bom modelo para este estudo porque apresenta um ciclo de vida curto e é de fácil manuseamento no laboratório.

Para obter a taxa de crescimento intrínseco foi necessário seguir a sobrevivência e a fecundidade das fêmeas de cada linha iso-fêmea. Os dados obtidos foram aplicados na equação de Euler-Lotka de onde foi possível retirar o valor de taxa de *r* para cada linha. Para obter *K*, dez fêmeas de cada linha foram inseridas num ambiente constante durante cinco gerações. O número de fêmeas ao fim deste tempo foi utilizado como *proxy* para a capacidade de suporte. Ao longo das gerações registaram-se ainda o número de fêmeas no início de cada geração, a fecundidade e a taxa de eclosão dos ovos. Para além do *r* e do *K*, mediram-se dois caracteres subjacentes potencialmente relacionados com os parâmetros demográficos: o uso individual de recursos e a interferência competitiva. O uso individual dos recursos foi obtido através da quantificação visual do dano infligido por uma fêmea em plantas de feijão durante 72 horas. Através da interferência competitiva foi possível perceber o quanto é que o uso individual dos recursos é afetado pela presença de competidores. Desta forma, para calcular a interferência competitiva, contabilizamos os danos infligidos por 4, 7 ou 10 fêmeas e comparamo-los com os danos causados por 1 individuo.

Os resultados obtidos demonstraram que as linhas iso-fêmeas apresentam variação genética entre si para todas as características medidas. Este é um resultado bastante significativo, pois vários modelos ecológicos negligenciam os efeitos da variação individual numa população, assumindo que todos os indivíduos apresentam as mesmas capacidades reprodutivas e de sobrevivência. No entanto, nenhuma correlação entre os parâmetros demográficos, ou entre estes e os caracteres subjacentes, foi encontrada. A ausência de uma correlação negativa entre *r* e *K* é concordante com o escasso suporte empírico na literatura para a teoria *r* e *K*. Apesar de não ter sido encontrada nenhuma correlação negativa entre os parâmetros demográficos e os caracteres subjacentes, obteve-se uma correlação positiva entre o uso individual de recursos e a interferência competitiva. Este resultado demonstrou estar de acordo com a teoria *r* e *K*, visto que esta diz que os indivíduos que são selecionados em *r* vivem num ambiente que permite o uso de quantidades abundantes de recursos devido à ausência de competição, no entanto indivíduos selecionados para *K* vivem em ambientes caraterizados por uma competição intensa que limita a utilização dos recursos. No entanto, os resultados sugerem que estes caracteres subjacentes poderão não ser representativos dos parâmetros demográficos visto que não foi encontrada nenhuma correlação entre estes dois níveis.

Os resultados deste projeto sugerem que *Tetranychus urticae* não é especialista em nenhuma estratégia, uma vez que na natureza esta espécie passa ciclicamente de uma fase de crescimento exponencial no início da colonização de uma planta nova, para uma fase de alta densidade e poucos recursos per-capita em poucas gerações. Adicionalmente, pode-se sugerir que a ausência de uma força significativa para selecionar as linhas apenas para uma ou ambas estratégias poderá explicar a variação entre os parâmetros demográficos nas linhas iso-fêmeas.

Palavras chave: Dinâmicas de populações; taxa de crescimento intrínseco; capacidade de suporte; correlações genéticas.

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Introduction

Populations are composed of several individuals that interact not only with other individuals of their own or different species but also with the environment. The result of these interactions will affect the demography of populations which, in turn, will affect the distribution of species across habitats. One essential demographic parameter of population dynamics is the intrinsic rate of increase – *r*. This parameter refers to the growth rate of a population that colonizes a new environment or is recovering from low density (Montero-Pau *et al.*, 2014). It thus measures the per capita growth rate regardless of the size of the population. (**Figure 1.1**) (Birch, 1948). This will result in an exponential growth that is described by the following equation:

$$\frac{dN}{dt} = r\,N \tag{1}$$

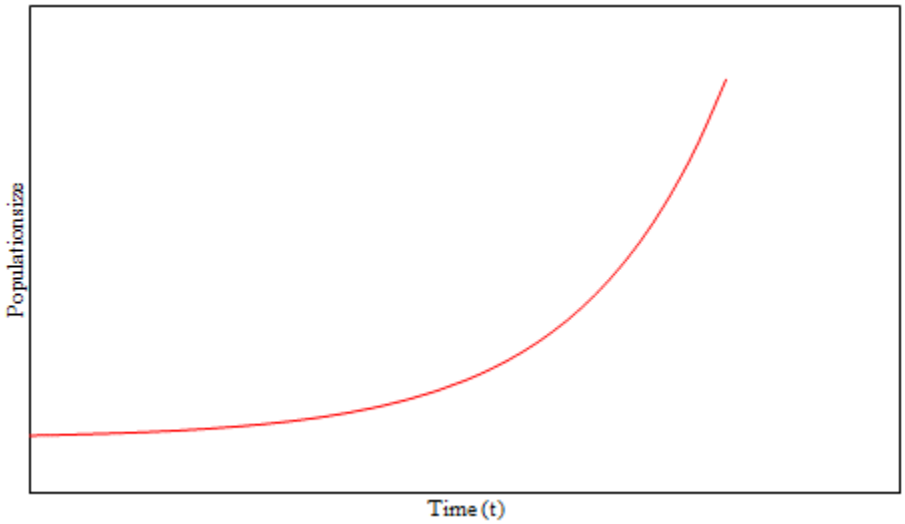


Figure 1.1- Graphical representation of the exponential growth of a population. If the intrinsic rate of a population is constant, the population will grow indefinitely. The X axis represents time while the Y axis represents the number of individuals in the population.

The intrinsic rate of increase can be estimated using the Euler-Lotka equation:

$$\Sigma\, l_x\, m_x\, e^{-rx} = 1 \tag{2}$$

in which l_x corresponds to the fraction of females surviving to age *x*, m_x to the number of individuals born at time *x*, and *r* represents the growth rate of the population. The equation gives the sum of the fecundity over the whole lifespan of the individuals, weighted by their age. Hence, this equation provides an estimation of how the population is growing based on the age of the females that compose it (Dublin and Lotka, 1925; Birch, 1948; Begon and Wall, 1987).

Since the intrinsic rate of increase does not consider limiting resources, population growth is exponential (Birch, 1948; Montero-Pau *et al.*, 2014). However, a population cannot maintain an exponential growth forever, as resources such as food availability or space become limited. This will generate a population growth that will be negatively affected by population density, which is described in the logistic model through the following equation (Verhulst 1838):

$$\frac{dN}{dt} = r\,(1 - \frac{N}{K}) \quad (3)$$

Under this model, at low densities, the population still grows exponentially. However, as densities increase, growth will decrease due to resource limitation, until it reaches an equilibrium, termed the carrying capacity – *K* (**Figure 1.2 – A**) (Begon, Townsend and Harper, 2006). The carrying capacity thus represents the number of individuals that the resources of an environment can sustain when population growth is zero (Dhondt, 1988).

In this model, the regulation of the population size is done via density-dependent birth and/or mortality rates (Taylor, Aarssen and Loehle, 1990; Begon, Townsend and Harper, 2006; Underwood, 2007; Iijima and Ueno, 2016). When the birth rate equals the death rate the population size reaches a stable point, the carrying capacity (Underwood, 2007; Iijima and Ueno, 2016). Below this point, the population will tend to increase since the birth rate is superior to the death rate, while above this point the population will tend to decrease because the death rate will surpass the birth rate (**Figure 1.2 - B**).

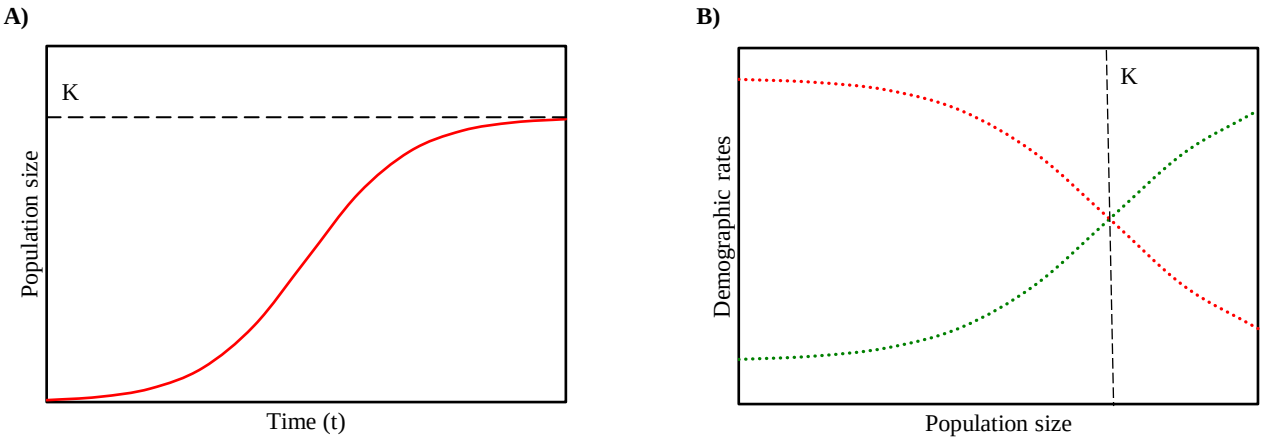


Figure 1.2- Representation of the growth of the population influenced by density-dependence. In A) the population size grows exponentially (*r*) over time until it reaches a point where the environment cannot sustain anymore population growth, which is defined as the carrying capacity (*K*). When reaching this point (defined by a population size), in B), the population mortality rate will surpass the birth rate, preventing the growth of the population. Figures inspired by Begon, Townsend and Harper, 2006.

When studying demographic parameters, intraspecific trait variation has been largely disregarded by both empirical and theoretical ecologists, generally that assumed that populations consist in identical individuals that present the same characteristics (Begon and Wall, 1987; Bjørnstad, Ottar N. and Hansen, 2013). Nonetheless, trait variation inside a population has been achieving a renewed appreciation (Bolnick *et al.*, 2011). Several studies have shown that individual variation can affect different population characteristics when compared to a homogeneous population, consecutively affecting deterministic and stochastic properties of the population process. For instance, it has been shown how individual variation generated by genetic and environmental sources can affect population dynamics (Vindenes, Engen and Sæther, 2008; Bolnick *et al.*, 2011) and also promote species coexistence (Begon and Wall, 1987).

To understand how individual variation affect the population dynamics through demographic parameters, it is necessary to understand how the demographic parameters and individual variation relate. Several theories have been proposed concerning the potential relationship between these parameters. The first, named *r* and *K* selection, is based on there being two contrasting types of environments which generate different selection pressures. In environments that are unpredictable in

time or short lived, individuals often experience periods of time that are favorable to a rapid population growth, allowing fast colonization of such unoccupied environments (Begon, Townsend and Harper, 2006). In such environments, mortality associated to high densities happens rarely, as those densities are seldom reached (Gadgil and Solbrig, 1972; Luckinbill, 1979; Parry, 1981). In this scenario, a population rarely reaches its carrying capacity and individuals are expected to be *r*-selected, which favors an allocation of the resources to reproduction (e.g., higher fecundity, early maturation) at the expense of body size and survival, which may ultimately affect competitive ability (Gadgil and Solbrig, 1972; Luckinbill, 1979; Parry, 1981). In contrast, individuals are expected to be *K*-selected in stable environments, in which resources are often limiting (Begon, Townsend and Harper, 2006). Individuals that live in such environments are continuously exposed to intraspecific competition, hence they experience density-dependent mortality (Reznick, Bryant, and Bashey 2002). Here, selection will promote the allocation of resources into traits that favor competitive ability, such as increased body size and survival. Individuals that invest many resources into somatic growth may do so at the expense of fecundity, which will, in turn, negatively affect the population growth rate (Gadgil and Solbrig, 1972; Luckinbill, 1979; Parry, 1981). In this way, the *r* and *K* selection suggests that there is a trade-off between the ability to colonize new habitats (related to the intrinsic rate of increase) and their competitive ability (related to their carrying capacity) (**Figure 1.3**). This means that weak competitors are strong colonizers, while strong competitors are weak colonizers (Limberger and Wickham, 2011; Vasseur *et al.*, 2011).

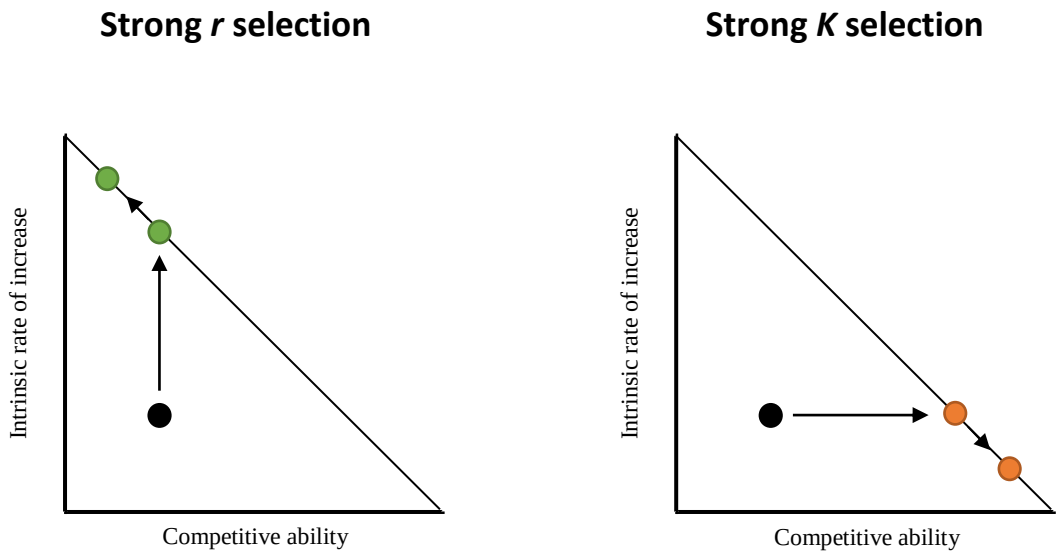


Figure 1.3- Representation of the colonization - competition trade-off. According to the *r* and K theory, it is expected that strong *r* selection in a population (black circles) will favor colonizers (green circles) at the expense of strong competitors, and strong *K* selection will result in the reduction of the colonizers due to competitive exclusion (orange circles). The black line corresponds to the maximum fitness possible (according to the *r* and *K* theory there is no possible genotype at the right of the line).

There is some empirical evidence for the existence of a trade-off between *r* and *K*. For example, after subjecting strains of *Drosophila melanogaster* to a *K*- and *r*-selection for eight generations, Mueller 1980 has shown that *K*-selected populations present higher per capita growth rate than the r-selected populations in high densities and the reverse was true at low densities (Mueller and Ayala, 1981). Also, Gadgil 1972 has shown an actual difference in the resource allocation in reproductive and non-reproductive traits in populations living in high density-independent and in high density-dependent environments, as described by theoretical expectations (Gadgil and Solbrig, 1972). However, these

empirical studies are scarce in the literature which challenges the generality of this theory (see Stearns, 1977; Clark, 1983).
The second hypothesis proposes that *r* and *K* are positively correlated. In Rueffler 2006, it shows how *r* and *K* increase linearly through the decomposition of the logistic equation (Rueffler, Egas and Metz, 2006):

$$\frac{dN}{dt} = (b - m)(1 - \frac{b-m}{\alpha} N), \quad (4)$$

from which we can conclude that:

$$r = (b - m) \text{ and } K = \frac{b-m}{\alpha} \quad (5)$$

In equations (4) and (5), b corresponds to the birth rate, m denotes the death rate, α is a constant that determines by how much the per-capita mortality rate increases with each increment in population size and N represents the population size. Through these equations it is possible to see that the carrying capacity may depend linearly on the traits that underlie the intrinsic rate of increase. This means that any variation in the intrinsic rate of increase could cause the carrying capacity to change proportionally, suggesting the existence of a positive correlation between *r* and *K*, rather than a trade-off (Clark *et al.*, 2003; Parvinen and Egas, 2004; Rueffler, Egas and Metz, 2006). Indeed, some empirical evidence suggest the occurrence of such positive correlation. For instance, Luckinbill 1979 has shown in *Paramecium primaurelia* that an *r*-selection increases the carrying capacity of the population, resulting in a strong positive correlation between the two parameters (Luckinbill, 1979). Also, Fitzsimmons 2010, when studying 65 strains of *Pseudomonas fluorescens*, has found that strains that presented higher intrinsic rate of increase showed higher carrying capacity, presenting a positive correlation (Fitzsimmons et al., 2010).

Lastly, equation (5) shows that *K* can change independently of *r*, if α varies, for instance with genetic of environmental factors, which gives rise to the hypothesis that *r* and *K* might not be correlated (Parvinen and Egas, 2004; Rueffler, Egas and Metz, 2006). This work suggests that looking at the traits underlying r and K might be necessary to fully understand the relationship of the two parameters. However, it still remains unclear to what extent individual behavior or life-history traits affect these demographic parameters. To date, no study has addressed this.

In this work we aim to test if the intrinsic rate of increase and the carrying capacity, two essential demographic parameters, present genetic variation and if they are genetically correlated, using the herbivorous spider-mite, *Tetranychus urticae*. This species is a good model to address this issue, for two main reasons. First, its populations are expected to face both unoccupied and highly occupied environments. Indeed, spider-mites often migrate to uninfested plants, where they rapidly start a new population (Krips *et al.*, 1998). However, these plants become crowded after a few generations, hence populations experience saturated environments (Wilson, 1987). Second, *Tetranychus urticae* has a short life cycle and is easily amenable to laboratory experiments. We will test potential correlations between *r* and *K* using isofemale lines of *Tetranychus urticae.* Specifically, we will test 1) if there is any genetic variance for the measured traits; 2) if the demographic parameters are correlated with each other; 3) if they are correlated with underlying traits; and 4) if these underlying traits are correlated with each other. Lastly, given the multifaceted and potentially large effect of trait variation in demographic parameters we will try to understand how this variation affect the demographic parameters correlations.

Material and Methods

Creation, expansion and maintenance of the isofemale lines

All experiments were performed using individuals from 18 isogenic lines. These lines were created from an outbred population of *Tetranychus urticae*, established in 2016 at the host laboratory, by merging six populations collected in the field around Lisbon in 2013 (**see Table 2.1**) (Zelé et al., 2018). Spider-mites were reared on 14-day-old bean plants. Bean was reared on a separate climate room to ensure an herbivore-free environment. All mites and plants used in the experiments were maintained under the same controlled conditions (the climate room was set to 24ºC, 60% of humidity and a light/dark ratio of 16:8 hours).

Table 2.1- Data from the 6 populations used to create the outbred population from which the isofemale lines were established

Name	Collection date	Collection Location	Coordinates	Host plant
TuDC	10/09/2013	S. Domingos	39.058742, -9.135427	Cucumber *(Cucurbita pepo)*
TuDF	10/09/2013	S. Domingos	39.058742, -9.135427	Bean *(Phaseolus vulgaris)*
TuCol	08/09/2013	Colares	38.799517, -9.448335	Bean *(Phaseolus vulgari)*
TuCH	31/10/2013	Casal Hortelão	38.851962, -9.393918	Tomato *(Solanum lycopersicum)*
TuRF	04/11/2013	Ribeira de Fráguas	39.366415, -8.851037	Tomato *(Solanum lycopersicum)*
TuAMP	18/11/2013	Aldeia da Mata Pequena	38.534363, -9.191163	Datura *(Datura stramonium)*

To generate isofemale lines, 250 mated females from the outbred population where individually placed on bean leaf discs of 2.55 cm2 Ø, placed inside a petri dish. Mites oviposited on those discs during 2 days, then were removed. 10 days later, the offspring reached adulthood and brothers and sisters mated with each other. Then, one mated daughter per line was transferred from the old disc to a new one, to start the next generation. Whenever the transferred female was newly-emerged, one brother was also transferred to ensure fertilization. This process was repeated every generation until an inbreeding coefficient of circa 0.94 was reached (i.e. 14 generations, see **SI.1 Table S.1**). Along this process, several lines were lost. At generation 14, we were left with 20 lines. Subsequently, all lines were expanded by transferring all individuals from each circle to a bean leaf (circa 7 cm length) in a Petri dish (9 cm diameter), containing water-saturated cotton. Line maintenance was performed under continuous generations: Lines were kept on leaves (circa 7 cm length) in a Petri dish (9 cm diameter), containing water-saturated cotton for 14 days. Subsequently, a piece of that leaf containing around 25 adult females was transferred to a new prepared Petri dish with a freshly cut bean leaf. After 72h, the oldest leaf was discarded and a new piece of leaf was added to the Petri dish to replace it to ensure resource availability. Water was added every 3 days.

To perform the experiments with same-aged females with the same mating status (i.e., virgin or mated), cohorts were created from 17 isogenic lines. For every cohort, 50 mated females from each isofemale line were collected from the maintenance petri dishes and isolated on cut leaves on a new petri dish. Females were allowed to lay eggs for 48 hours and were then killed. A newly cut leaf was added one week later to ensure resource replenishment. Water was added every 3 days. After one generation, each cohort generated more than 100 individuals, between 12 and 14 days old, which were directly used in the experiments described below.

Trait measurements

Intrinsic Rate of Increase - r

To measure the intrinsic rate of increase, we recorded the developmental time, lifetime age-specific survival and oviposition, and offspring sex ratio of single females. This experiment was performed on leaf discs (2.55 cm^2 Ø) that were cut from bean plants, placed in water-saturated cotton inside a squared Petri dish. Initially 19 lines were expected to be used in the experiment but at the time of the beginning of the experiment, only 17 isogenic lines presented high enough density to be used. A total of 20 replicates per isogenic line was performed (20 replicates * 17 lines = 340 replicates). From this number, 186 individuals were identified as females, of which only 107 females died of natural causes and were included in the analysis. Each female was allowed to oviposit for 6 hours, after which it was killed, and all eggs present in the disc but one, were randomly removed (**Figure 2.1- A**). Each patch was followed daily during the development of the egg and the time of emergence from the last molt before adulthood and the sex of the adult individual (tertiary sex ratio), or the timing of death, were recorded. On the 12th day, eggs that developed into males were discarded, while eggs developed into females were transferred to new leaf discs. From this point on, females were transferred every three days to a new leaf until death to ensure there were sufficient resources. During this period the survival of the females was tracked each day and fecundity was assessed every 3 days immediately after female transfer (See **Figure 2.1- B)**.

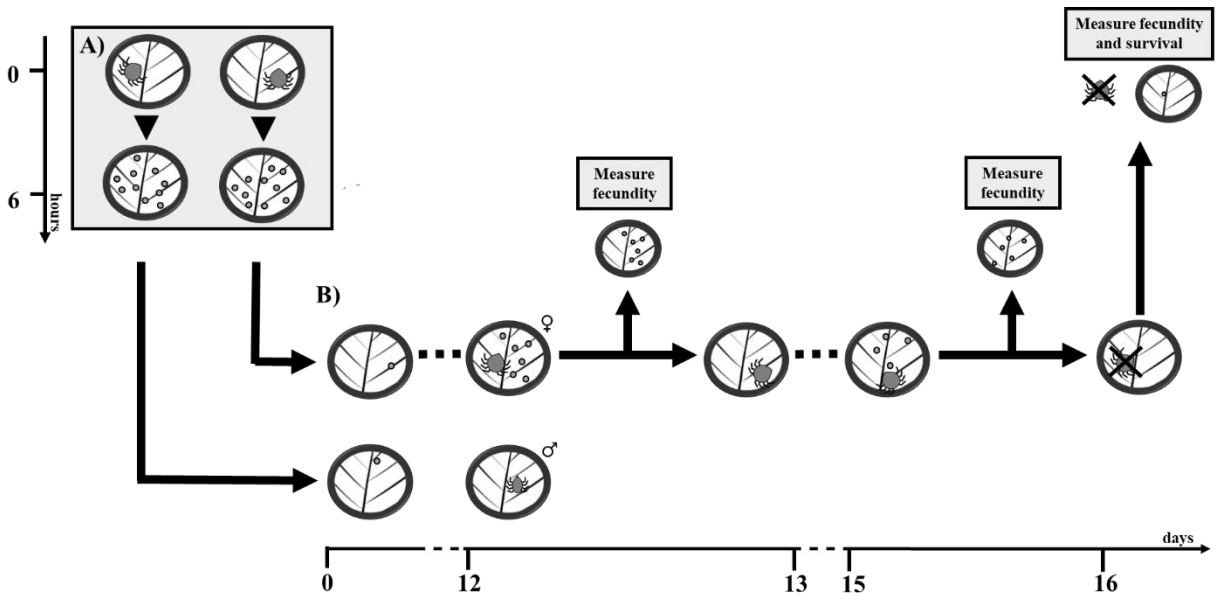


Figure 2.1 – Schematic representation of the protocol to assess the intrinsic rate increase. In A) a single mated female was allowed to oviposit for 6h on a leaf disc. Then in B), from the resulting eggs one was randomly chosen and its development was followed until death or adulthood. If the individual was a male the replicate was discarded by the 12th day. If it was a female, the individual was transferred to a new leaf disc where fecundity was counted until female death where the survival was obtained.

Carrying Capacity - K

Since this experiment required a considerable number of individuals simultaneously, the maintenance of the lines was adjusted so that bigger cohorts could be created. Specifically, the following modifications were done: a) in the first generation, instead of 25 mated females, 50 mated females were isolated onto a new container; b) instead of petri dishes with pieces of leaves, females were placed in a box (25cm x 17cm x 13cm) that contained a cup filled with water-saturated cotton with two cut bean plants; c) every week, the leaves of the old bean plant were placed on top of the new bean plant, allowing the passage of the individuals from the old leaves to the newer ones. To assess the number of individuals that can be sustained by a given amount of resource, i.e., the carrying capacity, 45 replicates of 10 mated females of each isogenic line (45 replicates * 17 lines = 765 replicates) were placed on a 12 mm^2 Ø leaf disc for 48h to oviposit and were then removed. The eggs obtained were counted and allowed to hatch and develop until adulthood. The number of juveniles was counted 5 days after female removal. Maintaining the individuals in the leaf disc for two weeks resulted in an environment with poor conditions that presented a challenge for the remaining individuals. 12 days after female removal, the surviving individuals were counted and sexed, and the females were transferred to a new disc to start the next generation (See **Figure 2.2**). The same protocol was performed for 5 generations, each time using the surviving females of the previous generation to found the following generation. We expect that populations would reach an equilibrium density by then, which would be detected by the number of females resulting from the leaf disc being similar to the one from which the population on that disc was initiated.

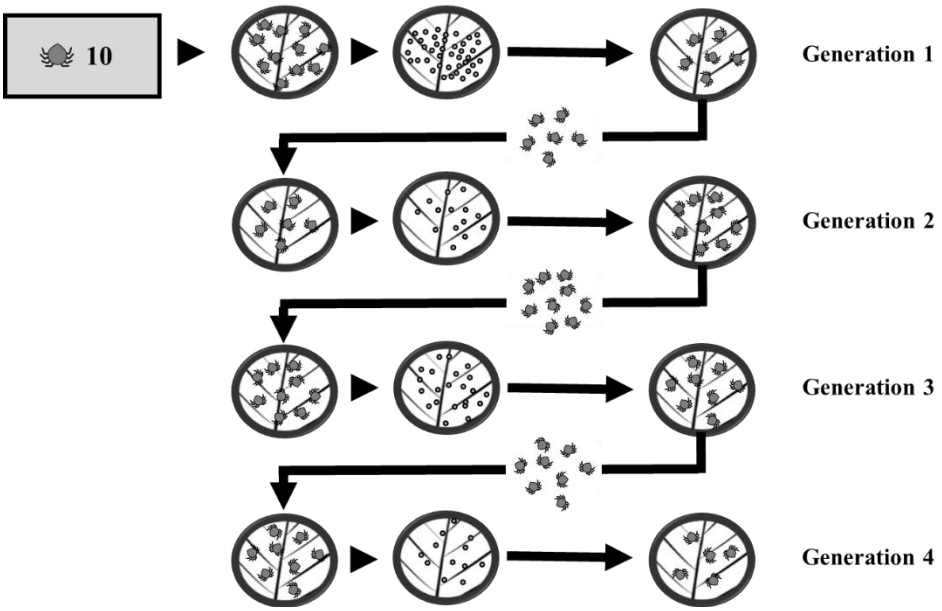


Figure 2.2 – Representation of the experimental procedure to assess the carrying capacity. To initiate the first generation 10 females were placed on a leaf disc where they remained for 2 days in order to oviposit. Females were removed and the eggs present in the leaf disc were counted and allowed to develop until adulthood. The number of individuals that hatched from the eggs and that survived until the 14th day was counted and the females alive were transferred to a new leaf disc where the new generation started.

Underlying traits – resource use and competitive interference

To measure the resource use of each line and how it is affected by the presence of competitors, we quantified the damage inflicted to leaf discs by one, four, seven and ten female spider-mites. However, for the purpose of this study, only the results of the damage done by one and ten individuals were used in the analysis (12 replicates per density were done to all isofemale lines). Same aged, mated adult females were collected from the cohorts at day 14 and placed on a leaf disc inside of a Petri dish for 72 hours. Females, eggs and any kind of debris were removed from the leaf disc, prior to the image analysis. Spider-mite consumption is easily noticeable since it leads to the formation of chlorotic spots that are associated with an extensive collapse of the mesophyll layer, displaying an yellowish color on the leaf (**Figure 2.3**) (Bensoussan *et al.*, 2016).

All images were taken using the stereo microscope Zeiss Stereo Lumar v.12 present in the FCUL Microscopy Facility. This microscope presents a high contrast and color fidelity that is necessary to spot the spider-mite damage. The settings of the image acquisition, used for all images, were the following: Exposure= 1/34; Gain= 18-22; Sharpness= 7; Zoom= 9.6x; Contrast= 15; Brightness= 30. The leaf disc was placed with a flat-head tweezer on a Petri dish without cotton in order not to damage the leaf and a thin square of glass was placed on top of the leaf to flatten the leaf disc. Each image captured ¼ of the leaf disc, so each leaf disc was divided into 4 images that were later merged into one using the software Adobe Photoshop. To measure spider-mite damage, we used an objective high-throughput standardized screening method, and processed the images using two software programs: First we used Ilastik, an artificial intelligence processing program that segments and classifies two-dimensional surfaces based on labels provided by the user (Sommer *et al.*, 2011). To ensure a successful segmentation we trained the software Ilastik to recognize mite damage on the leaf circles. This was done through the characterization of 4 distinct labels that correspond to a specific type of tissue found in the images obtained: one for the background; one for the veins; one for the leaf mesophilum; and lastly one for the spider-mite damage. Several discs were used to train Ilastik by manually selecting each one of the labels and marking the corresponding areas. After the training was complete, the software automatically segmented every leaf disc image. We then used ImageJ, to quantify the pixel area of each segmentation obtained in Ilastik.

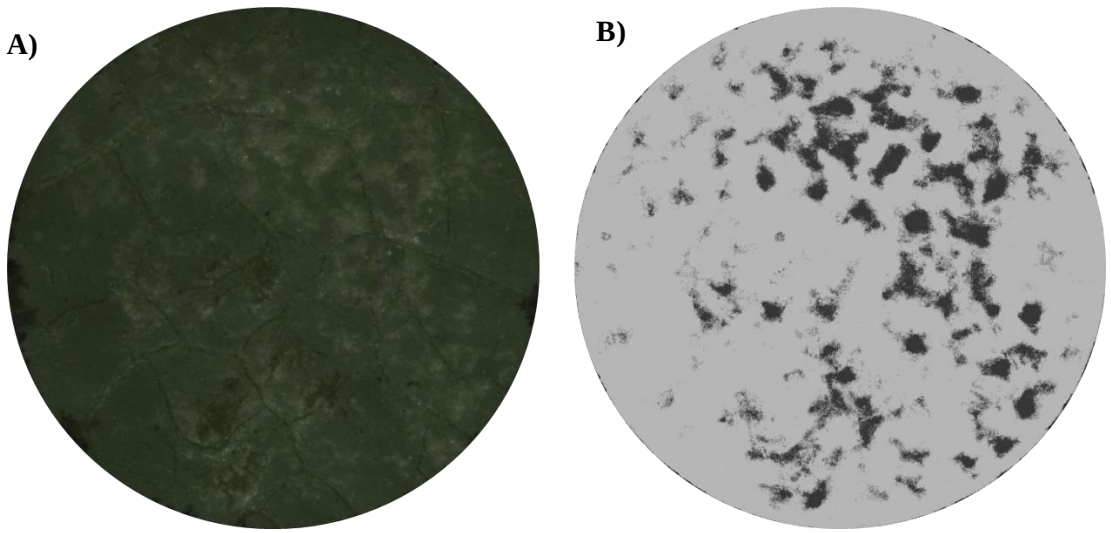


Figure 2.3 – Example of leaf damage inflicted by 7 females. As seen A) before and B) after the segmentation, from B), ImageJ will then determine the percentage of damage done in pixels to the leaf disc.

Since the damage done by the spider-mites presents a color similar to the veins in the leaf disc, when identifying the damage spots, the software Ilastik® sometimes selected also larger leaf veins. Through a visual inspection, replicates were excluded when this conflict was evident (arrow in **Figure 2.4**).

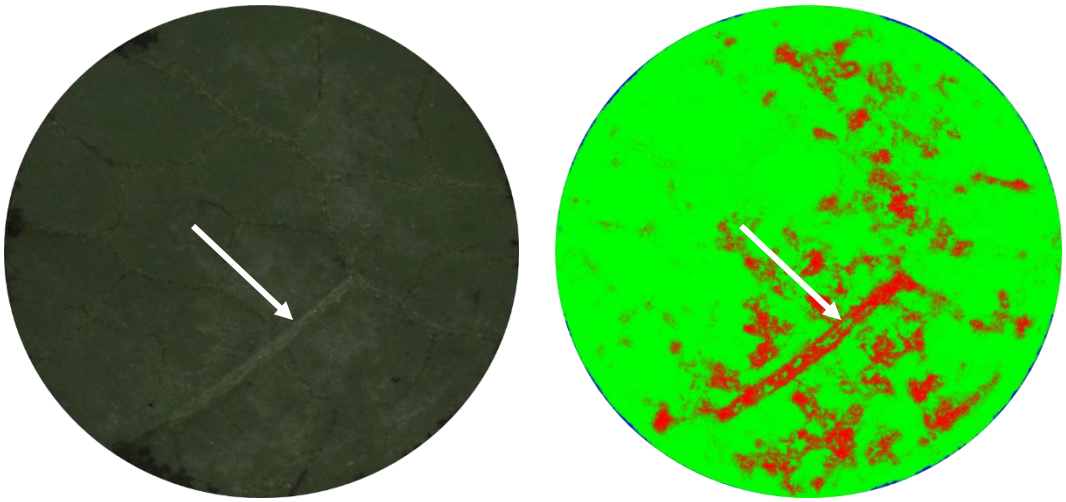


Figure 2.4 – Example of a vein being mistaken by damage. Ilastik® segmenting a leaf vein as damaged area.

With the data obtained in the experiment, we estimated the following variables:

1) The resource use, measured as the damage area done to the leaf circle by a single individual;
2) The competitive interference, obtained through the following equation:

$$\text{Competitive Interference} = \frac{rU * N - dN}{rU * N} \quad (6)$$

Where *rU* corresponds to the area damage done by a single individual, *N* denotes the number of individuals in the competitive scenario and *dN* corresponds to the area of damage done in the competitive scenario. Multiplying *rU* by *N* provides an estimate of how much damaged area *N* individuals would do in a non-competitive scenario. This way, it was possible to measure how much the resource use of each isofemale line is affected by the presence of competitors.

Individual traits

The description of the methods used for these experiments is described in **SI2**.

Statistical analysis

All statistical analyses were performed with the software RStudio (version 3.2.5, R Development Core Team 2016).

Trait variance among lines

To obtain a confidence interval for the intrinsic rate of increase, a bootstrap with 10.000 replicates was performed. Note that this parameter is estimated from the sum of values of several individuals, hence 'classical' standard deviation cannot be calculated, as we have obtained a single value per line. This has proven to be an obstacle when calculating variance. We thus analyzed the variance of the underlying traits that composed the intrinsic rate of increase, namely fecundity and survival under ad libitum conditions. Both models were fitted with line as a fixed factor and day as a random factor. To test differences in fecundity among isofemale lines, a generalized mixed-effect model (glmer, lme4 package) was performed. To test differences in survival, a cox proportional hazards mixed-effect model (coxme, coxme package) was performed.

In the carrying capacity experiment, the analysis of the number of females through the generations was done in 3 steps:

1) Including all generations – to check whether the number of females varied across generations;
2) Between the last two generations – to check if there was a stabilization of the number of individuals in the two generations, which could indicate that the carrying capacity was reached;
3) For the last generation (if the carrying capacity was reached) – to check if there were differences among the lines, which would indicate differences in *K*.

In all three analyses, since the values were greatly overdispersed, we performed a generalized linear mixed model with a beta-binomial error distribution (glmmTMB package) (Skaug et al. 2013). Furthermore, in order to understand how the carrying capacity was reached (only per individual mortality or by the combination with other factors), the fecundity and the hatching rate were also analyzed. The fecundity in the carrying capacity experiment was Box-Cox transformed to improve normality (Crawley 2007) and subsequently analyzed using a linear mixed-effect model (lmer, lme4 package). The hatching rate was analyzed performing a cbind for the relative proportions of unhatched eggs and hatched eggs and using a generalized linear mixed model with a beta-binomial error distribution to account for overdispersion (glmmTMB package) (Skaug et al. 2013). In the analysis of number of females including all generations and the between the last two generations, line, generation and their interaction were used as fixed factors while day was used as random factor. The same analysis was repeated for every trait (number of females, fecundity and hatching rate) but only for the last generation with only line as fixed factor and day as random factor.

All the models used to analyze the resource use contained line as fixed factor and day as random factor. The area of damage of resource use of the isofemale lines was Box-Cox transformed in order to improve normality (Crawley 2007) and then analyzed through linear mixed effect models (lmer, lme4 package). To analyze competitive interference, a generalized linear mixed model with a beta-binomial error distribution to account for overdispersion (glmmTMB) was performed.

Genetic correlations among traits

As pointed before, since the intrinsic rate of increase is a single value per line, there was no variation for this trait (the variation obtained in the bootstrap was not used in order to prevent the pseudo-replication of the results). Therefore, the correlation coefficient between the intrinsic rate of increase and the remaining variables was determined by performing a Pearson Correlation (Adler and Parmryd, 2010).

Genetic correlations with all other variables were done using a multivariate model (MCMCglmm package) (Hadfield, 2010). The phenotypic variance and its covariance of all traits analyzed were estimated using a bivariate MCMCglmm model with "line" and "day" as random effects. Covariances were rescaled as correlations and were supported when a 95% credible interval excluded zero. Priors for the reported models were slightly informative and generated by partitioning the phenotypic variance evenly among each random term and were given a low degree of belief (V =diag(n) * Vp/r, nu =2; Hadfield, 2010). Convergence was evaluated by visually inspecting plots of the model parameters and assessing autocorrelation values. The posterior distribution of the models was sampled every 300.000 iterations after a burn-in period of 500 iterations for a total of 10 samples (Taylor *et al.*, 2012).

Results

Intrinsic rate of increase

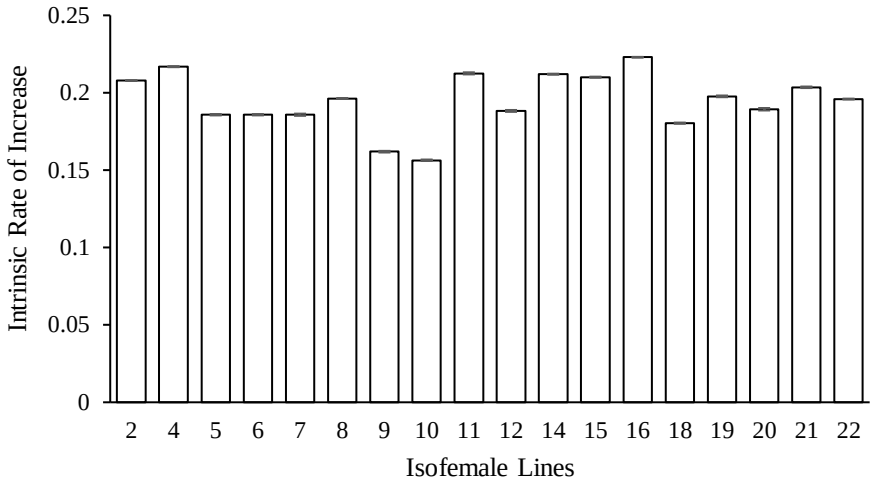


Figure 3.1 – Average intrinsic rate of increase in the isofemale lines of *T. urticae*. Each bar represents the average intrinsic rate of increase per isofemale line. Vertical bars correspond to standard errors of the mean.

The intrinsic rate of increase varied across lines (**Figure 3.1**). When decomposing this trait into its parts, we found that the isofemale lines differed significantly in their total fecundity ($F_{1,17}$ =170.48 p <0.001; **Figure 3.2**), but not in their female survival (X^2_4 =23.30 p =0.14; **Figure 3.3**).

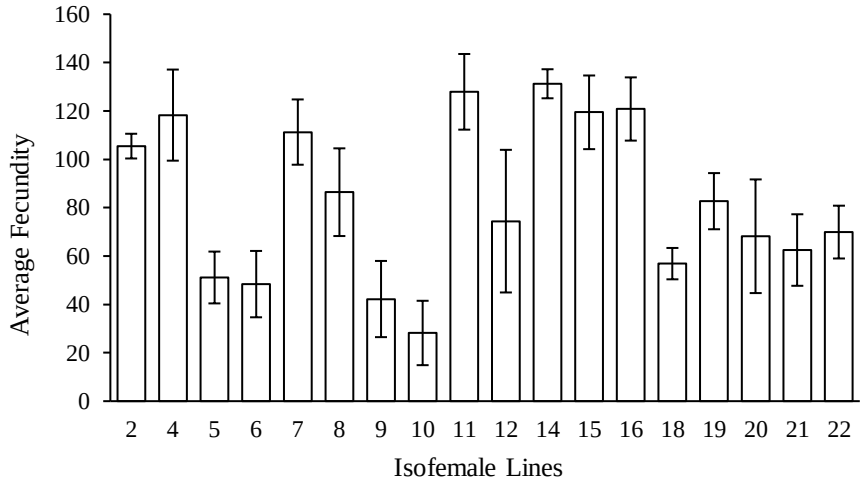


Figure 3.2 – Average fecundity per isofemale line in the intrinsic rate of increase experience. Each bar represents the average fecundity per isofemale line. Vertical bars correspond to standard errors of the mean.

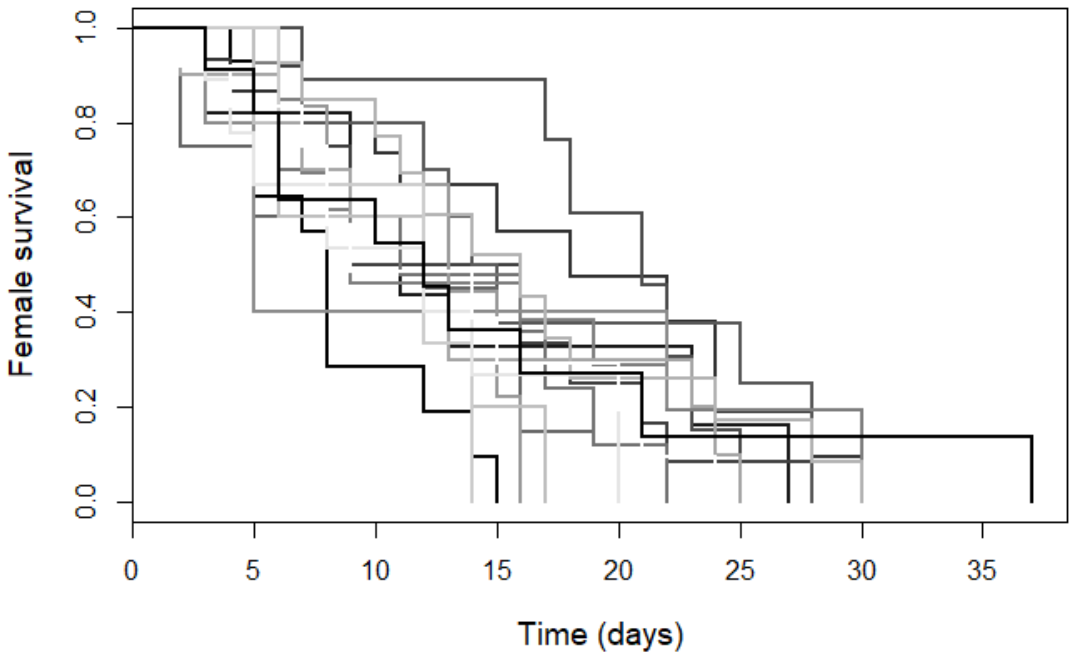


Figure 3.3 – Average female survival of the isofemale lines of *T. urticae*. Each line represents the average probability of the females from an isofemale line to be alive at a given time point.

Carrying Capacity

When analyzing the data for the last generation only (**Figure 3.4**), assuming this was the generation the carrying capacity was reached, we found that the number females presented significant differences among isofemale lines ($F_{1\text{-}17}$ =2.38 p =0.001; **Figure 3.4- A**) but the number of eggs laid and the hatching rate showed no significant differences among isofemale lines ($F_{1\text{-}17}$ =1.51 p =0.08 and X^2_{19} =21.03 p=0.21 respectively; **Figure 3.4- B and C**).

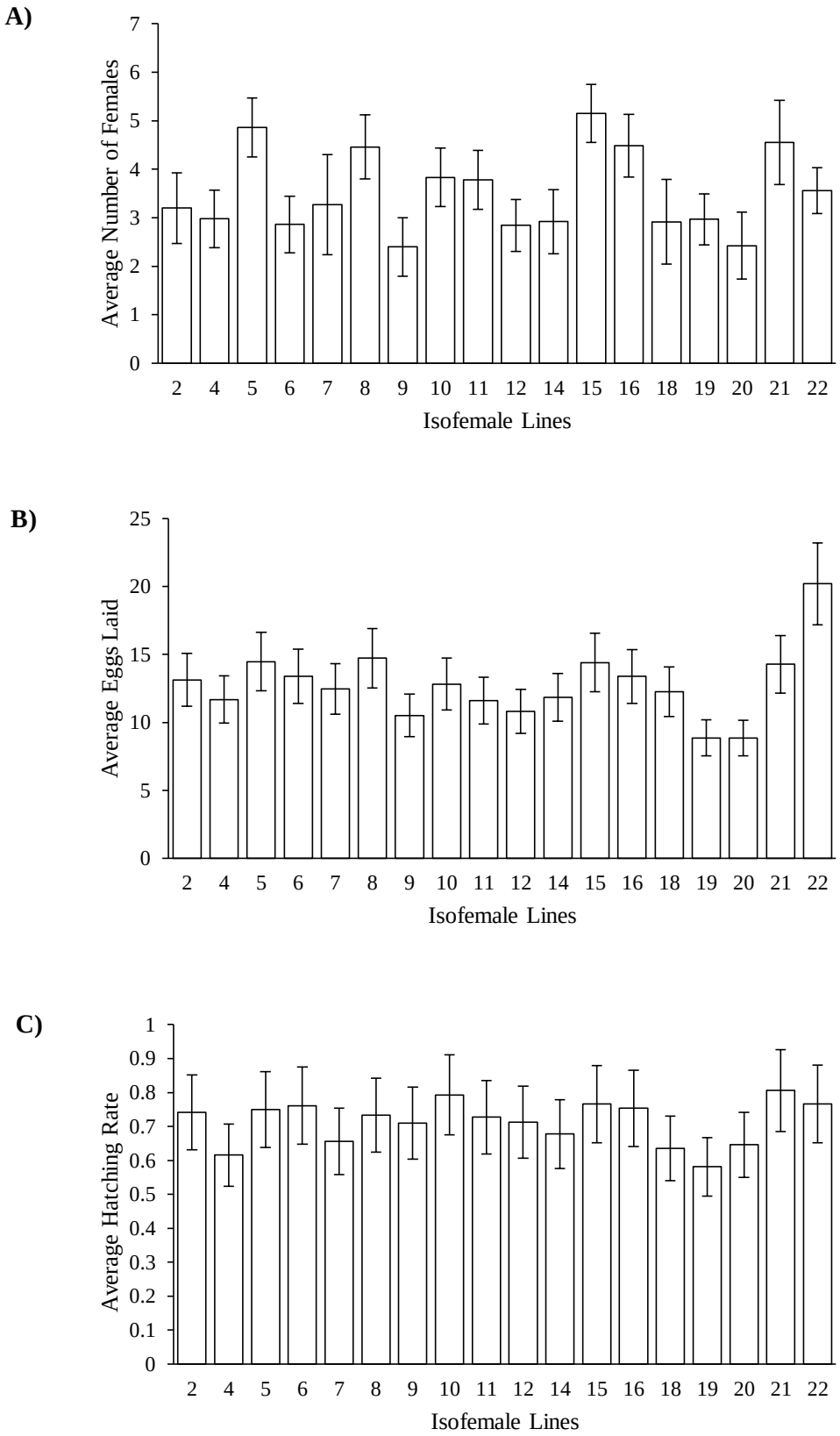


Figure 3.4 - Average number founder females (A), of eggs (B) and proportion of eggs hatched per patch (C) at the last generation. In A) it is possible to see the average number of females in the fifth generation while B) and C) contain the average eggs laid and average hatching rate in the fourth generation respectively. Vertical bars correspond to standard errors of the mean.

The number of *T. urticae* females per patch at the beginning of each generation was significantly different across the five generations (X^2_1 =39.22 p <0.001; **Figure 3.5**) but this variation was not significantly different across isofemale lines (X^2_{17} =16.04 p =0.52). However, when only analyzing the last two generations, significant differences between isofemale lines were found (X^2_{17} = 39.20 p =0.001). Furthermore, the number of females founding these last two generations did not differ significantly (X^2_1 =0.01 p =0.89), suggesting the number of females could be used as a proxy for the carrying capacity.

The average number of eggs laid per leaf disc was significantly affected by the interaction between isofemale line and generation ($F_{1\text{-}17}$ =32.40 p =0.01; **Figure 3.6**). However, the isofemale lines presented no significant difference among them ($F_{1\text{-}17}$ =1.38 p=0.13), while generations presented a significant difference ($F_{1\text{-}17}$ =192.01 p <0.001). The hatching rate was not significantly affected by the interaction between isofemale line and generation (X^2_{17} =19.10 p =0.32; **Figure 3.6**), nor by the isofemale lines alone (X^2_{17} =27.30 p =0.06). However, there was a significant effect of generations (X^2_1 =123.47 p<0.001).

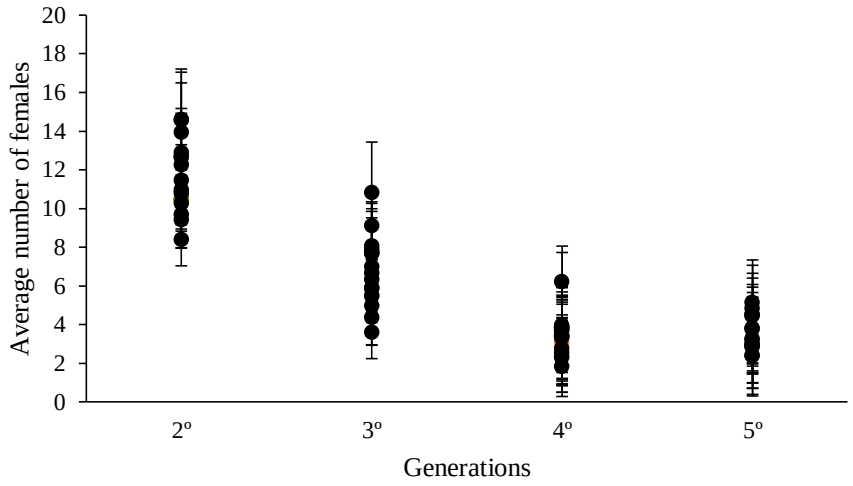


Figure 3.5 - Average number of females at the start of each generation. Each marker corresponds to the average number of females for each isofemale line. Vertical bars correspond to standard errors of the mean.

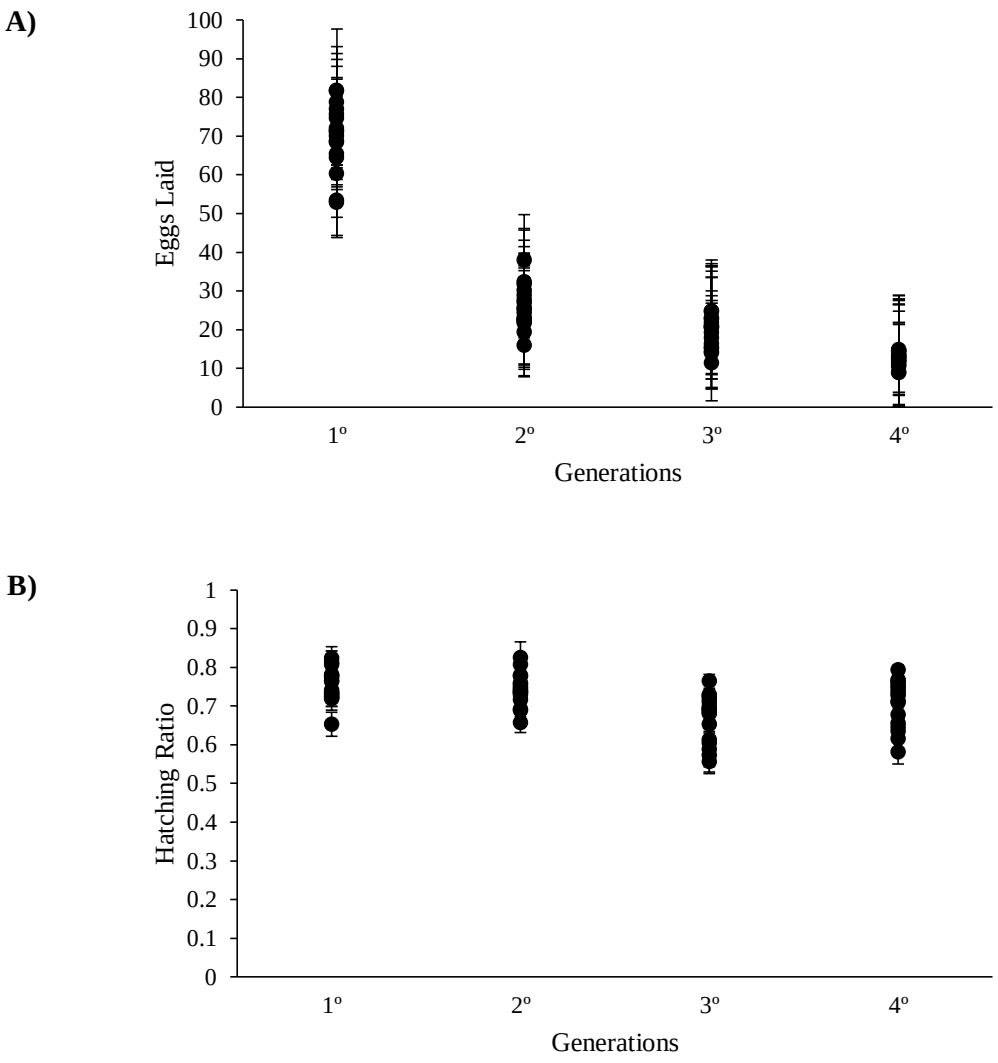


Figure 3.6 – (A) Average number of eggs laid across the generations and (B) average hatching ratio through the generations. Each marker corresponds to the average value of eggs laid (A) or hatching ratio (B). Vertical bars correspond to standard errors of the mean.

Damaged leaf area

All traits measured from damaged leaf area, i.e., the resource use and competitive interference, were significantly different across isofemale lines ($F_{1\text{-}17}$ =133.08, p <0.001 **Figure 3.7 - A**; $F_{1\text{-}17}$ =194.16, p<0.001 **Figure 3.7 – B**).

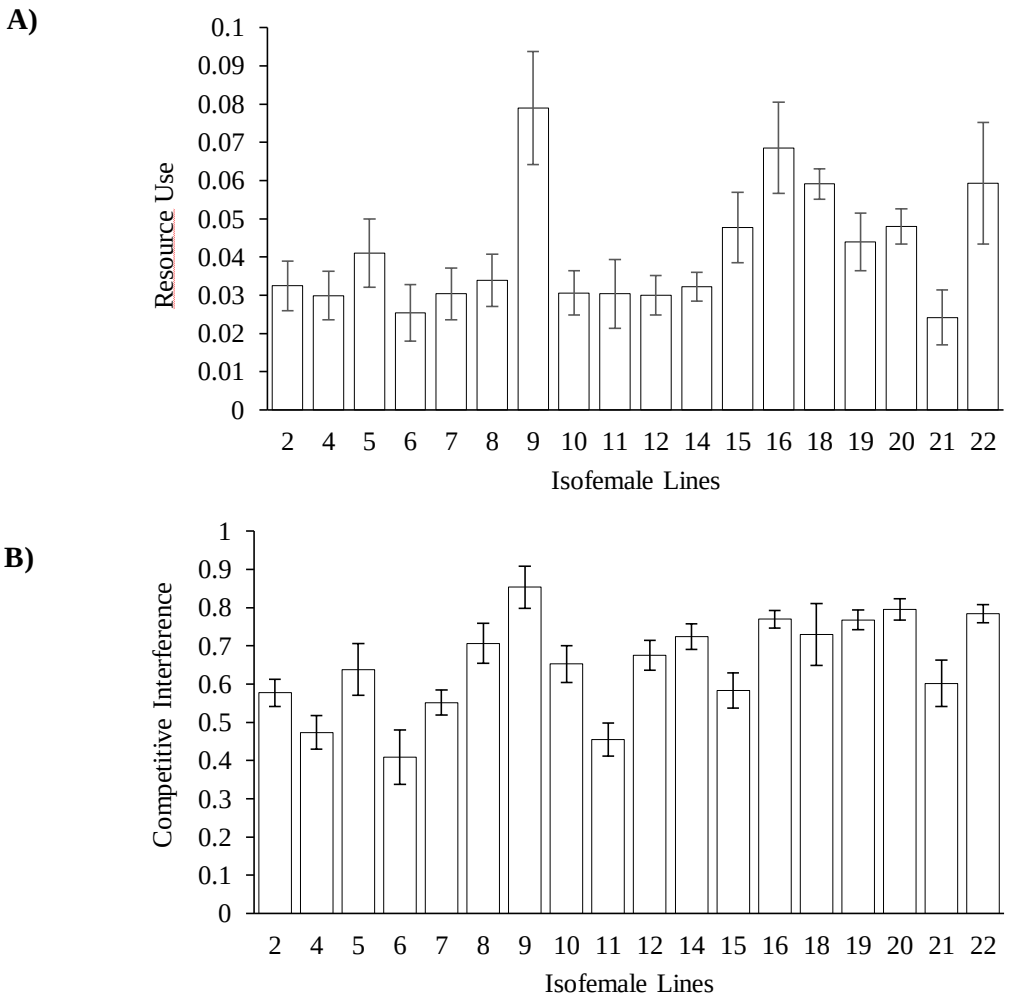


Figure 3.7 - Average Resource Use and Competitive Interference. In A) is represented the average percentage damaged area to every isofemale line caused to a leaf disc by only one female and in B) it is represented in percentage how much the average resource of the isofemale lines is affected by the presence of 9 competitors. Vertical bars correspond to standard errors of the mean.

Genetic Correlations

No significant correlations were found across isofemale lines between the intrinsic rate of increase and (i) the number of females alive at the fifth generation (correlation =0.30, 95% percent confident interval=-0.21 to 0.68, p =0.24; **Figure 3.8**); (ii) the resource use of one female (correlation =-0.12, 95% percent confident interval= -0.57 to 0.38, p =0.64; **Figure 3.9- A**); (iii) interference caused by the presence of competitors (correlation =-0.40, 95% percent confident interval =-0.74 to 0.10, p =-0.40; **Figure 3.9- B**). The result of the correlations with the individual traits is presented in the *supplementary information* (**Figures SI.3 Table 1 and Figure 1**).

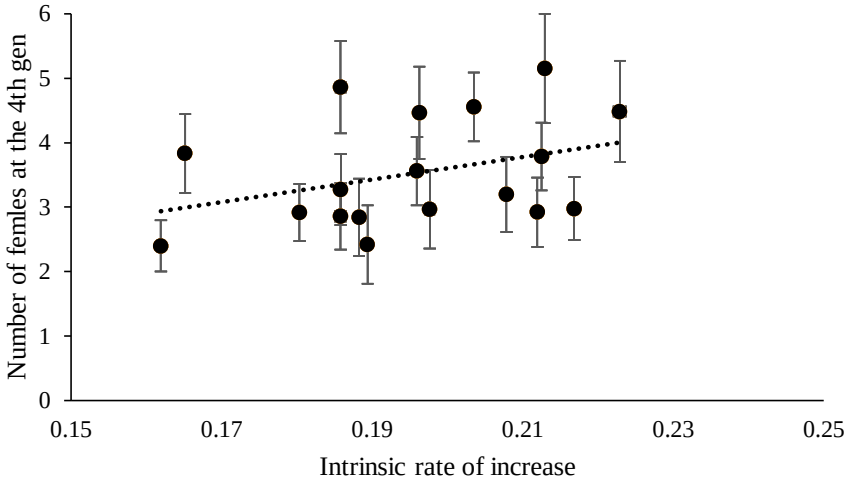
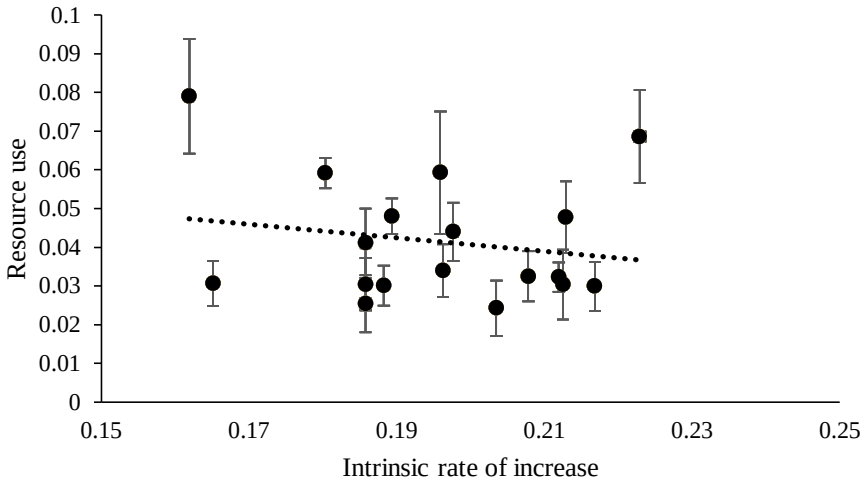


Figure 3.8 – Correlation between the intrinsic rate of increase and the number of females at the fifth generation. Pearson correlations between the intrinsic rate of increase and the number of females in the fifth generation (the carrying capacity).

For the remaining combinations (**Table 2; Figure 3.10)**, only a positive correlation between resource and competitive interference (rmat =0.70 and confident interval =0.21 to 0.88; **Figure 3.11**) was detected. All other traits were not significantly correlated. Indeed, we did not detect any significant correlation between the number of females alive at the fifth generation and the remaining parameters (resource use: rmat =-0.09 confidence interval =-0.58 to 0.39; competitive interference: rmat =-0.18, confidence interval =-0.60 to 0.41). The result of the correlations with the individual traits is presented in the *supplementary information* (**Figures SI.4 Table 1 and Figure 1**).

A)



B)

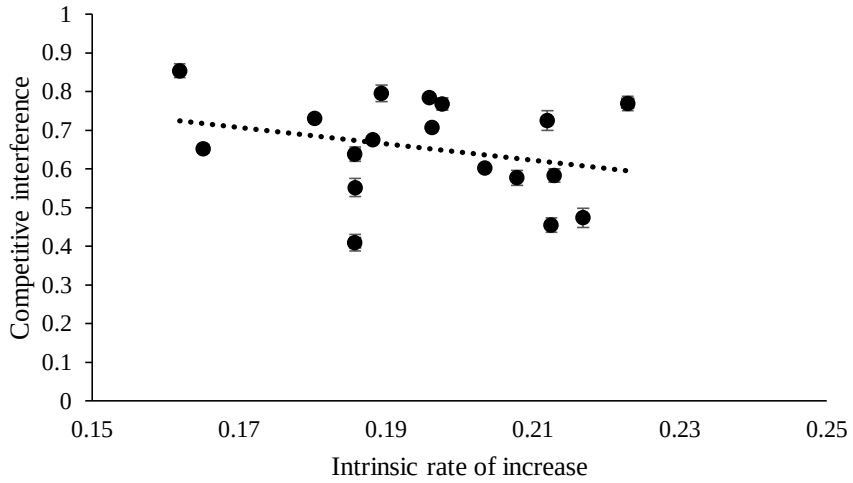
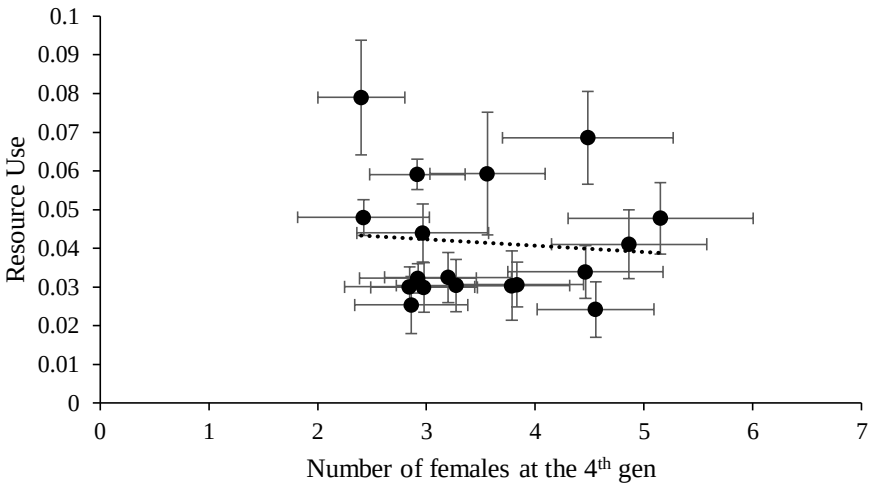


Figure 3.9 – Correlations involving the intrinsic rate of increase. Pearson correlations between the intrinsic rate of increase and (A) the average resource use; (B) percentage of competitive interference in each isofemale line.

A)



B)

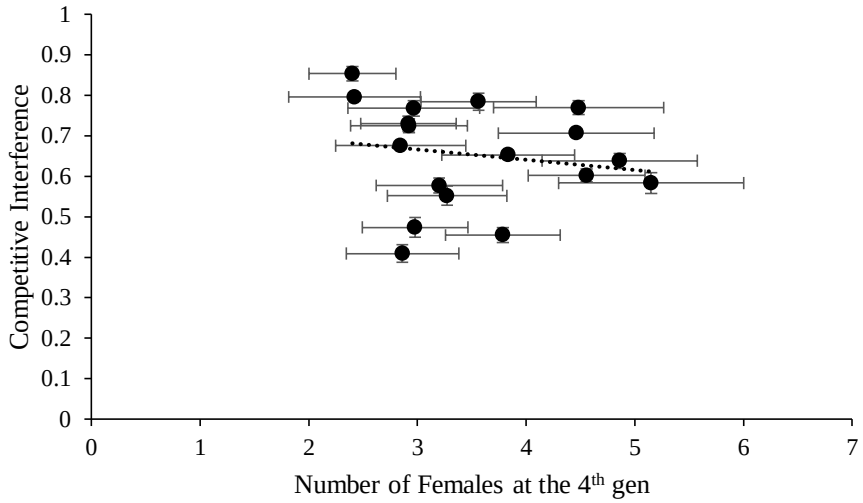


Figure 3.10– Correlations involving the carrying capacity. Graphical representation of the correlations between the carrying capacity and (A) the average resource use of the isofemale lines; (B) percentage of competitive interference of the isofemale.

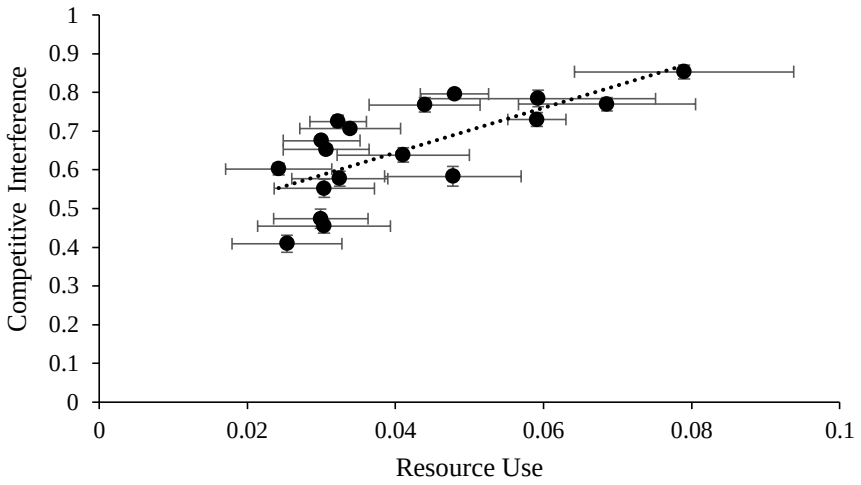


Figure 3.11 – Correlation between the resource use and the competitive interference. Graphical representation of the correlations between resource use and the competitive interference.

Table 3.1 - Genetic correlations using the MCMCglmm model. Genetic correlations of two traits with the carrying capacity in the *T. urticae* isofemale lines. Correlations that were different from zero and supported by a 95% credible interval that excluded zero are indicated in bold. The upper triangle (dark gray) contains the value of the correlation between the traits and in the lower triangle (light gray) covariances. Correlations and covariances that were different from zero and supported by a 95% credible interval that excluded zero are indicated in bold. In the diagonal (no color) are indicated the variances and were calculated as the mode of the posterior distribution with 95% credible intervals and are bounded above zero.

Genetic Correlations			
	Females Alive	Resource Use	Competitive Interference
Females Alive	0.32 (0.10 to 0.60)	-0.09 (-0.58 to 0.39)	-0.08 (-0.60 to 0.41)
Resource Use	-0.02 (-0.14 to 0.10)	0.13 (0.05 to 0.24)	**0.70 (0.21 to 0.88)**
Competitive Interference	-0.02 (-0.15 to 0.11)	0.15 (-0.10 to 0.41)	0.36 (0.12 to 0.65)

Discussion

This project aimed at testing different theories that predict how two demographic parameters, the intrinsic rate of increase and the carrying capacity are correlated and affected by underlying traits. Using 17 isofemale lines of *T. urticae*, we showed that all demographic parameters and the underlying level traits were genetically variable. However, we found no significant correlation between: (a) the demographic parameters and (b) the demographic parameters and the underlying and individual traits. We did, however, find a positive correlation between the two underlying traits, resource use and competitive interference.

Ecological dynamics before stabilization

In the carrying capacity experiment, even though the amount of resource per capita increased throughout the generations (due to a reduction in the number of females), the fecundity decreased gradually as generations went by, after an initial increase in the first generation. This might be explained by the presence of maternal effects, where the conditions experienced by the parents affects the offspring performance in a non-genetic manner (Mousseau and Fox, 1998). Indeed, the females used to start the first generation grew in an environment in which resources were abundant before being exposed to resource-limiting conditions, whereas the females of the following generations were continuously exposed to the resource limiting environment. Also, we found that fecundity changed across generations, suggesting that the stabilization in the number of individuals might depend, even if partially, on this trait. Regarding the hatching rate, a significant difference was also found across generations, which could imply that the conditions where the parents are present may also affect the quality of the eggs. However, this trait varies much less than fecundity across generations, suggesting that density dependence affects fecundity more than egg quality.

The stabilization in female numbers between the two last generations suggests that the carrying capacity was attained. However, to make sure this was indeed the case, the number of generations performed should be increased.

Genetic variation

Regarding the intrinsic rate of increase, the variance in the isofemale lines for this trait was inferred through the two life-history traits used to calculate it, fecundity and survival. The isofemale lines differed significantly in fecundity but they did not show any differences in survival. This result suggests that a higher fecundity might not have a negative effect in the lifespan of the females, as shown in other studies with *T. urticae* (Magalhães *et al.*, 2007). In addition, it suggests that the differences observed in the intrinsic rate of increase are mainly due to the genetic variance in fecundity among lines. Concerning the carrying capacity, although we found genetic variation for this trait (measured as differences among isofemale lines in the last generation), we did not find it for fecundity or hatching rate in that generation. Hence, possibly another life-history trait (e.g., mortality) accounts for genetic variation in *K*.

Population ecology generally rests upon the assumption that all individuals present the same characteristics (Bolnick *et al.*, 2011). This way, most ecological models assume that individuals in a population have the same capacity of survival and reproduction even though it is known that natural populations are composed by individuals with different characteristics (Vindenes, Engen and Sæther, 2008). Such potential variation between organisms of the same population has crucial consequences for ecological and evolutionary dynamics (Bolnick *et al.*, 2003, 2010; Vindenes, Engen and Sæther, 2008; Agashe, 2009). However, how much variation there is in demographic parameters it is not entirely clear. Most studies concern the effect of environmental variation in *r* and *K* (e.g., Underwood 2004, 2007), with a few exceptions (see Luckinbill, 1979; Mueller and Ayala, 1981). Since our results show a

significant difference across the isofemale lines for each trait, there is potential for these to be genetically correlated.

Genetic correlations

We found no correlation between *r* and *K*. In addition, the absence of genetic variation for longevity rules out the possibility of a genetic correlation between this trait and fecundity, a result in accordance with that of Magalhães *et al.*, 2007. Such result goes against the *r* and *K* theory, which predicts that individuals that invest in reproductive traits should show reduced survival, or vice-versa (Luckinbill, 1978, 1979; Parry, 1981). The absence of a negative correlation in this work also corroborates the lack of empirical support for the *r* and *K* selection theory in the present literature. For instance, Luckinbill applied *r*-selection to a mixed population of *Paramecium* strains to test if there was a reduction of the carrying capacity described in the *r* and *K* theory. However, instead of a reduction of the carrying capacity through *r*-selection, the results showed a positive correlation between the intrinsic rate of increase and the carrying capacity (Luckinbill, 1979).

One possible explanation for the absence of a negative genetic correlation is revealed when we decompose the demographic parameters to an underlying level. According to equation 5 of the introduction, *K* is a direct function of *r* (Mallet, 2012). This is proved by some studies, for instance, Kuno who has shown that a variation in the carrying capacity due to temperature differences was promoted through a variation in the intrinsic rate of increase, generation a positive correlation (Kuno, 1991). In another study, when low doses of toxic chemicals are added to water environments, the carrying capacity of several species is reduced along with their intrinsic rate of increase (Hendriks et al 2005).

However, we found that isofemale lines that presented higher resource use also presented higher values of competitive interference. This would corroborate the trade-off between *r* and *K*. If these underlying traits were representative of the demographic traits, a positive correlation between the resource use and the intrinsic rate of increase would be expected, since the amount of resources invested in reproduction could translate into a proportional increase of the intrinsic rate of increase. This relation also occurs in 3 species of hydras submitted to two rates of feeding. In this study, it was shown that the intrinsic rate of increase was positively affected by the rate of feeding (Stiven, 2016). Additionally, several studies have described how the amount and quality of food positively influences the fecundity of the organisms (Wrensch and Young, 1975; Kjesbu *et al.*, 1998) which in turn could affect the intrinsic rate of increase. Nonetheless, we did not detect any correlation between resource use and the intrinsic rate of increase. Furthermore, since *K*-selected individuals live in environments characterized by a low amount of resources per-capita due to high densities, a negative correlation between the resource use and the carrying capacity could be expected, but instead no correlation was found. Furthermore, since a *K*-environment selects individuals with better competitive abilities due to densities that approach the carrying capacity (Taylor, Aarssen and Loehle, 1990), this should translate into populations that present higher values of carrying capacity having a lower sensibility to competition. Consequently, since the competitive interference corresponds to how the presence of competitors affect the resource use of a focal individual, isofemale lines with higher carrying capacity should, according to the *r* and *K* theory, have lower competitive interference. However, once again, no correlation was found. Finally, we could also expect the intrinsic growth rate and the competitive interference to be positively correlated, considering that *r*-selected individuals live in environments characterized by a potentially weak effect of competition (Gadgil and Solbrig, 1972; Parry, 1981; Begon, Townsend and Harper, 2006). However, this correlation was not found. Similarly, no correlation between *K* and resource use was found.

These results may be explained by the analysis of the damage done to the leaf not being an appropriate proxy for the amount of food the individuals acquire. Another possibility could be the existence of variation in the assimilation efficiency between the isofemale lines, which would mean that the amount of food ingested does not translate directly into higher population growth rates. Even though a positive correlation between the resource use and the competitive interference was found, these traits seemed to be uncorrelated to the intrinsic rate of increase and the carrying capacity. Therefore, this result cannot be used to corroborate the occurrence of a trade-off between *r* and *K*.

Furthermore, the absence of a correlation between *r* and *K* can happen via an effect of α, a constant that determines the amount of increase in per-capita mortality rate with each new single individual added to the population (Parvinen and Egas, 2004; Rueffler, Egas and Metz, 2006). Thus, the absence of correlation between *r* and *K* found in this study suggests that the genetic variation present among isofemale lines for the carrying capacity is promoted by changes in α (Mallet, 2012). To better understand this, we can decompose α into $a * l$, in which *a* is the amount of resources exploited by a focal individual and *l* represents the sensitivity of an individual to the addition of an extra individual (see **Figure 4.1**).

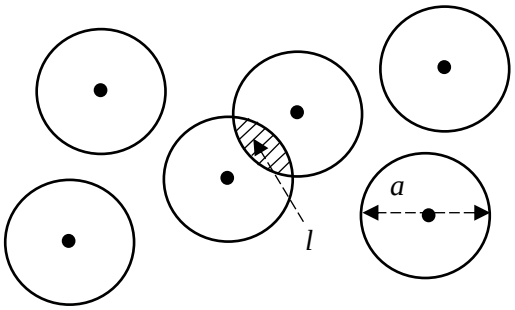


Figure 4.1 – Neighborhood interaction. In this figure it is represented how the increase of conspecific individuals affect the competition neighborhood of the focal individual. The circle represents the amount of resource required for each individual. The circle overlapping represents the conflict between the resources used by both individuals.

Since *l* describes how an individual is affected by the increment of each individual added, the trait measured that best matches it is the competitive interference. Likewise, *a* has a direct correspondence to resource use measured here. Therefore, if α is varying across the isofemale lines, through $K = \frac{r}{\alpha}$, it is possible to explain the absence of a positive correlation between the intrinsic rate of increase and the carrying capacity.

Additional tests – individual traits

Considering that *r* and *K* are demographic parameters, their selection should depend upon several characteristics of the individuals. We thus tested for potential correlations between these parameters and other traits that we had previously measured using these same isofemale lines. . Firstly, it has been shown that the body size of the individuals can affect both demographic parameters in opposing ways. Since larger individuals are usually *K*-selected (Pianka, 1972), we could expect body size to be negatively correlated with *r* and positively correlated with *K*. However, no correlation was found (**SI3 Figure S1**). Second, *K* could also increase with increasing values of mating competitive ability, and *r* could decrease due to the allocation of resources in competitive traits (Ovadia and Schmitz, 2002; Branson, 2008). However, no correlations were found between the competitive mating rate and the demographic parameters. Our results failed to prove the existence of a selection of individual traits based

on the demographic parameters stated in the theory of *r* and *K*. Nonetheless, future experiements using different underlying traits could prove the existence of a selection.

Conclusions

With this project, it was possible to understand that the isofemale lines presented variation for every measured trait. Our study highlights the importance of ecological studies having in consideration the variation among individuals of the same population, since this variation affect several aspects of the population dynamics. Furthermore, the absence of a correlation between *r* and *K* and between the demographic parameters and the underlying traits suggest that the *r* and *K* selection might not act as described theoretically.

Spider-mites often colonize new environments. However, as their populations expand rapidly, they also often reach a saturation in the environment. Then, after some time, the spider-mite population will disperse again to less crowded environments. Therefore, spider-mites go from a phase of exponential growth when colonizing a new environment to a phase of high population density and high competition due to the low amount of resources per capita available. Thus, *Tetranychus urticae* may not benefit from being specialized in any strategy, which could also help explain the maintenance of variation for traits associated to *r* and *K* in the isofemale lines.

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Supplementary information

SI.1 – Coefficient of inbreeding

Table S1 In this table it is described for each generation the coefficient of inbreeding (IC) and the probability of having a fully inbreed line (Pi).

Generation	*Pi*	*IC*
0	0.000	0.000
1	0.000	0.000
2	0.125	0.250
3	0.250	0.375
4	0.375	0.500
5	0.484	0.594
6	0.578	0.672
7	0.656	0.734
8	0.721	0.785
9	0.773	0.826
10	0.816	0.859
11	0.851	0.886
12	0.880	0.908
13	0.903	0.926
14	0.921	0.940
15	0.936	0.951
16	0.948	0.961
17	0.958	0.968
18	0.966	0.974
19	0.973	0.979
20	0.978	0.983
21	0.982	0.986
22	0.986	0.989
23	0.988	0.991
24	0.991	0.993
25	0.992	0.994
26	0.994	0.995
27	0.995	0.996
28	0.996	0.997
29	0.997	0.997

SI.2- Protocols of the individuals traits experiences

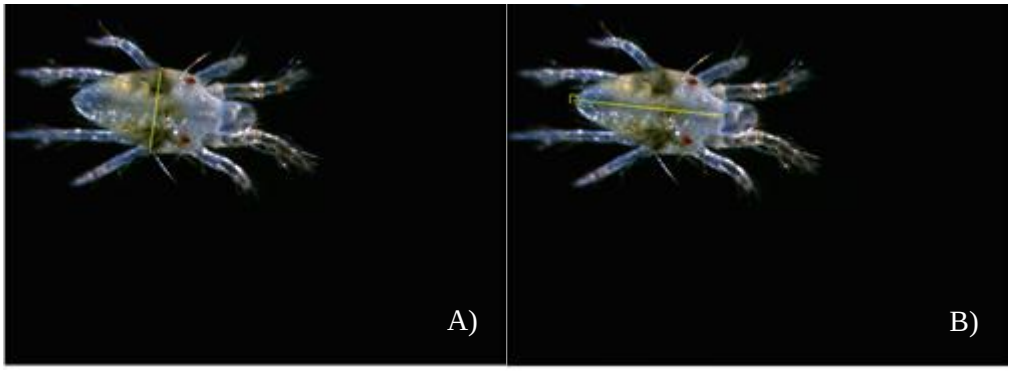
Cohorts to obtain males

Similarly to the cohorts described in ***Materials and Methods***, to ensure that all the males used in the experiments had the same age, cohorts of virgin females were created. To do it so, 25 quiescent females were collected from each isofemale line and placed in a new petri dish prepared as previously described. Since spider-mites are haplodiploid, these virgin females will produce unfertilized eggs that will give rise to males. The maintenance of these cohorts followed the same schedule as that of the mated female cohorts and they generated 12- and 14-days old males that were used in the experiments described below.

Male size

The males were collected from the cohorts one day before the adult stage. In order to measure the size of the males of each isofemale line a small drop of water was placed on the middle of the surface of a Petri dish. A single individual was placed on top of the drop of water, so that its body was parallel to the petri dish and its limbs were easily seen. After the preparation of the individual to measure, the Petri dish was placed on the magnifier *Zeiss Stereo Lumar* v.12 for image acquisition with the following settings: Exposure= 1/34; Gain= 18-22; Sharpness= 7; Zoom= 120x; Contrast= 15; Brightness= 30. In order to obtain the area of the individuals, two different measurements were done: 1) the length of the idiosoma and 2) the width of the idiosoma, connecting the third legs. The area was calculated through: Area= average length * average width * π, and was obtained in μm^2. All the measurements were performed through the software ImageJ.

Figure S.1- Example of the measurement of the length (A) and width (B) of the idiosoma in a male *Tetranychus urticae*.



Competitive ability

A male from each line was placed in a leaf disc with a competitor male from an independent population and a virgin female from a different line. The leaf disc was observed until the first mating or for a maximum of one hour and the first male to mate with the female was registered. To distinguish the males, they were painted with water colors a few hours before observation

Re-mating behavior

The re-mating behaviour was measure as the time a male takes before mating with a mated female. To obtain this, a male from each line was placed in a leaf disc where there was a mated female from a different line, created from another independent population. These females were collected from a cohort of mated female created as described in the main text. This patch was observed until mating occurred or for a maximum of 2h and the timing was registered using a stopwatch.

Mating rate

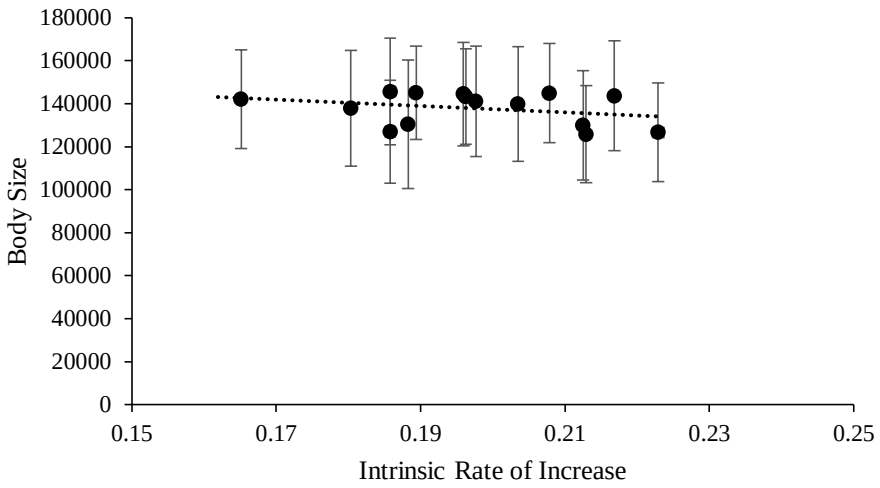
For this experiment, the males obtained from virgin females cohorts of each isofemale line were collected two days before emergence. The females used in the experiment were collected from mated female cohorts from another independent population. A male from each line was placed in a leaf disc with eight virgin females and the number of females a focal male mated with, during one hour, was registered using a stopwatch.

SI.3- Results of the correlation between the intrinsic rate of increase and the individual traits

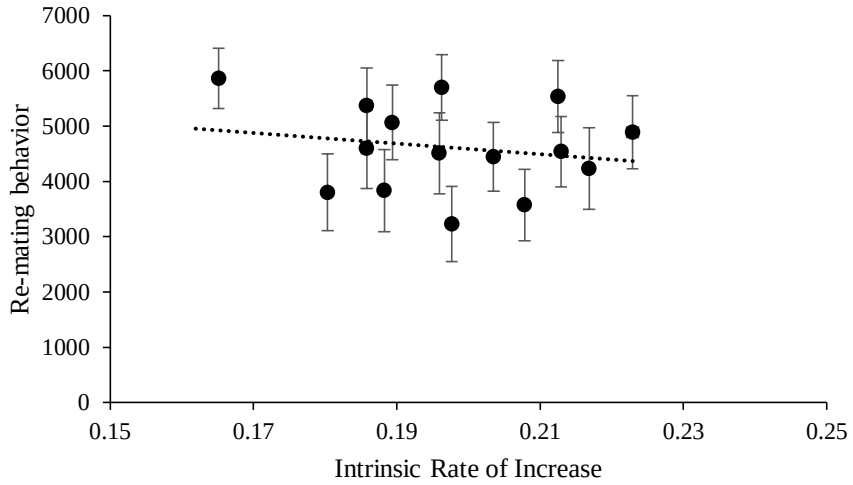
No significant correlations were found across isofemale lines between the intrinsic rate of increase and (i) the body size (correlation =-0.20, 95% percent confident interval=-0.66 to 0.36, p =0.48); (ii) the re-mating behavior female (correlation =0.008, 95% percent confident interval= -0.52 to 0.53, p =0.94); (iii) the mating competitive ability (correlation =0.15, 95% percent confident interval =-0.41 to 0.63, p =0.60); and (iv) the mating rate (correlation =-0.12, 95% percent confident interval =-0.57 to 0.38, p =0.64).

Figure S.1– Correlations involving the intrinsic rate of increase. Graphical representation of the correlations between the carrying capacity and (A) the body size; (B) re-mating behavior; (C) mating competitive ability; (D) mating rate.

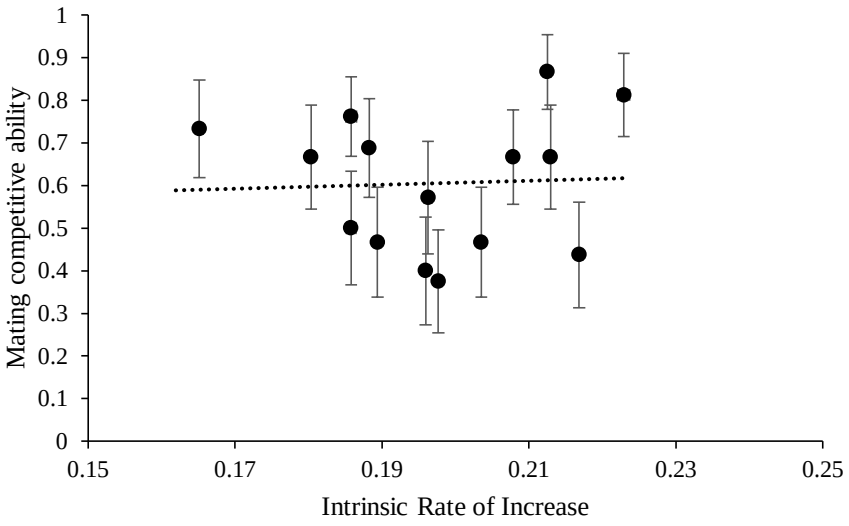
A)



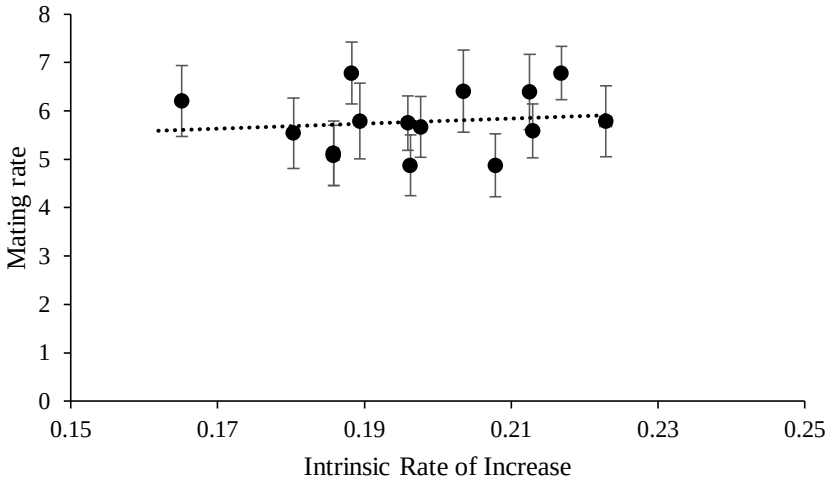
B)



C)


Mating competitive ability
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.15
0.17
0.19
0.21
0.23
0.25
Intrinsic Rate of Increase

D)


Mating rate
8
7
6
5
4
3
2
1
0
0.15
0.17
0.19
0.21
0.23
0.25
Intrinsic Rate of Increase

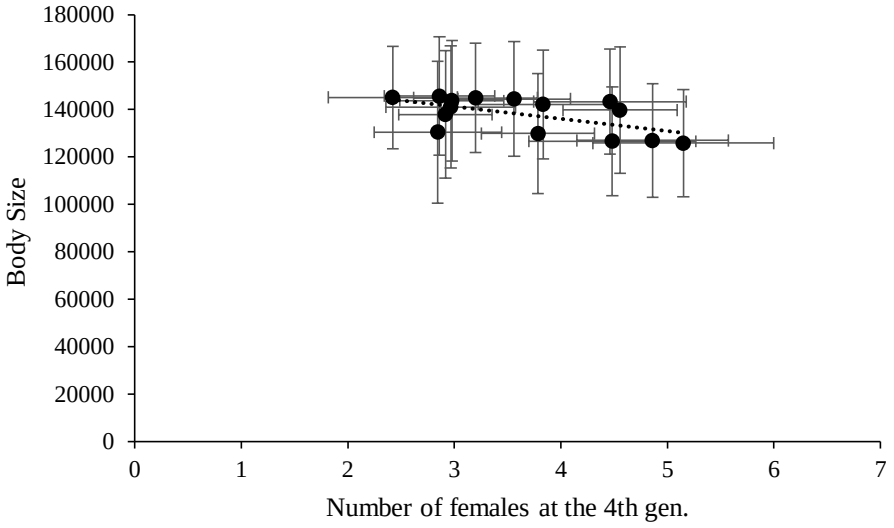
SI.4- Results of the correlation between the carrying capacity and the individual traits

Table S1 - Genetic correlations using the MCMCglmm model. Genetic correlations between the number of females alive at the fifth generation; the resource use; and the competitive interference with the individual traits. This genetic correlations were done using the multivariate model (MCMCglmm package). Correlations that were different from zero and supported by a 95% credible interval that excluded zero are indicated in bold.

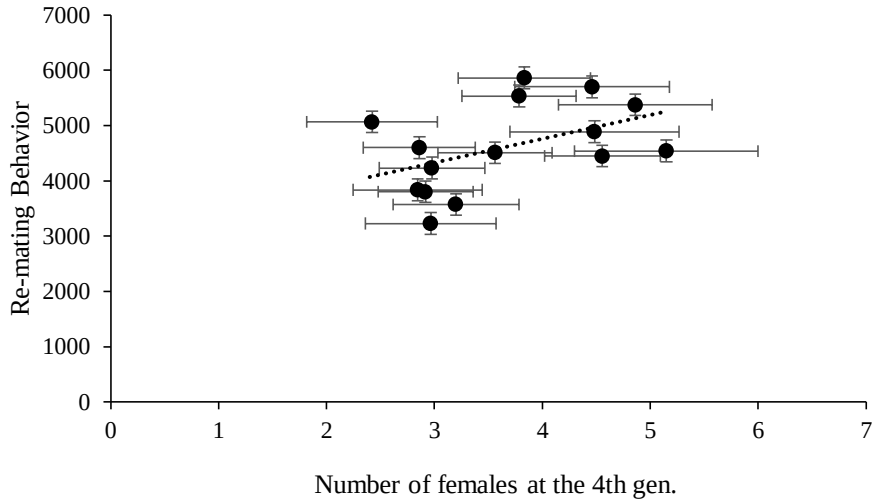
Genetic Correlations				
	Male Size Area	Competitive Ability	Re-mating Behavior	Mating Rate
Females Alive at the 4th gen	-0.31 (-0.70 to 0.20)	-0.05 (-0.51 to 0.47)	0.14 (-0.40 to 0.60)	-0.10 (-0.52 to 0.45)
Resource Use	-0.25 (-0.73 to 0.32)	-0.08 (-0.58 to 0.54)	0.001 (-0.60 to 0.51)	-0.007 (-0.61 to 0.48)
Competitive Interference	0.003 (-0.52 to 0.52)	-0.26 (-0.72 to 0.30)	-0.14 (-0.58 to 0.49)	-0.01 (-0.57 to 0.49)

Figure S.1– Correlations involving the carrying capacity. Graphical representation of the correlations between the carrying capacity and (A) the body size; (B) re-mating behavior; (C) mating competitive ability; (D) mating rate.

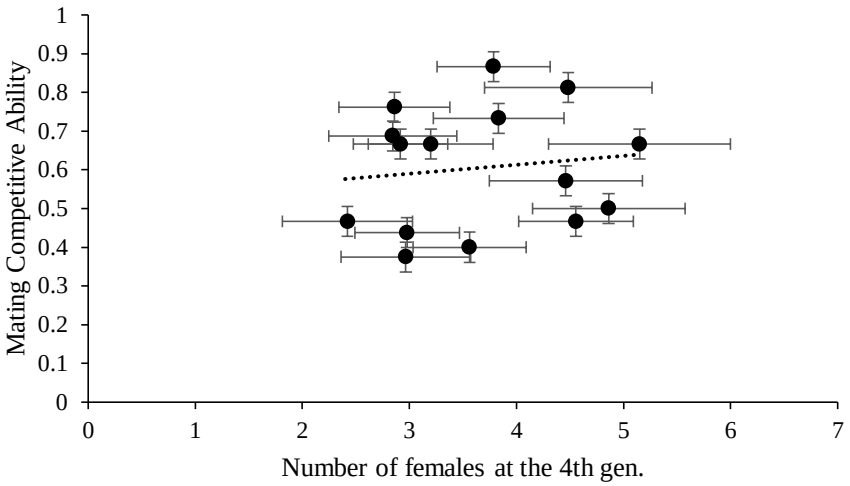
A)



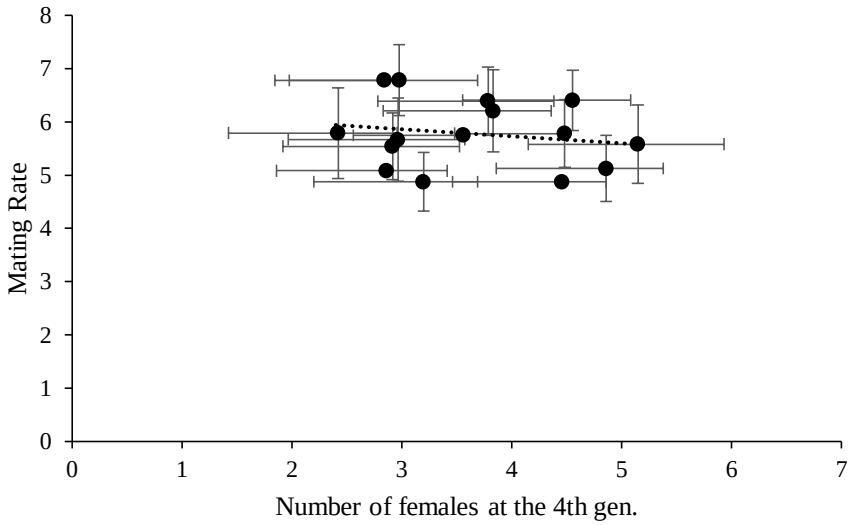
B)



C)


Mating Competitive Ability
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
1
2
3
4
5
6
7
Number of females at the 4th gen.

D)


Mating Rate
0
1
2
3
4
5
6
7
8
0
1
2
3
4
5
6
7
Number of females at the 4th gen.